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TITLE: **Evaluating the efficacy of ERG targeted therapy in vivo for prostate tumors**

PRINCIPAL INVESTIGATOR: **Phuoc T. Tran, MD, PhD**

CONTRACTING ORGANIZATION: **Johns Hopkins University, Baltimore, MD 21231**

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13. SUPPLEMENTARY NOTES

14. ABSTRACT The proposed research will examine the suitability of ERG as a target for prostate cancer therapy by using novel transgenic mice. Prostate cancer is a large health problem in the United States. Recent efforts to classify distinct molecular subtypes of prostate cancer have shown that >50% of prostate cancers possess a chromosomal translocation involving the *ERG* oncogene. I hypothesized that ERG can serve as an effective molecular therapeutic target for prostate tumors using novel prostate tumor mouse models. During this third year of support we have not been able to adhere to our "Statement of Work" - Task#2 or Task#3. We were successful at completing Task#1, but characterization of ERG expression from our prostate inducible mouse model did not demonstrate any detectable prostate specific ERG expression at the protein level. Data from another project using the *ARR2PB-tTA* line has lead us to believe that the level of expression from the *ARR2PB-tTA* line is low and perhaps insufficient for the *in vivo* experiments described in our proposal. To remedy this issue with low prostate specific expression we proposed to re-start Task #1 of the project with the new prostate specific TET driver mouse, *Hoxb13-rtTA*, but difficulties with breeding have hampered our progress. We have overcome these issues and have been able to see robust prostate specific expression using this *Hoxb13-rtTA* driver. We are now in the process of reinitiating our studies on the ability of ERG to collaborate with *AKT1* with these new mice, *Hoxb13-rtTA*/ *tetO-ERG.*

15. SUBJECT TERMS
ERG, prostate cancer, inducible transgenic mouse model

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Evaluating the efficacy of ERG targeted therapy in vivo for prostate tumors
PI – Phuoc T. Tran, MD, PhD

INTRODUCTION:

The proposed research program will elucidate the role of *ERG* in prostate cancer and the suitability of this gene as a target for therapy by using novel modular inducible transgenic mice. Prostate cancer is the most common cancer diagnosed in men in the United States. It has been estimated that greater than 200,000 new cases of prostate cancer were diagnosed in the United States in 2012 and prostate cancer was responsible for ~30,000 deaths or the second most common cause of cancer deaths in men (*1*). Recent efforts to classify distinct molecular subtypes of prostate cancer have led to the novel findings that greater than 50% of prostate cancers possess a chromosomal translocation involving the *ETS* oncogene family of transcription factors (*2, 3*). These *ETS* translocations result in dysregulated overexpression of the *ETS* oncogene in prostate cancer cells. The most common ETS family member involved in these translocation events is the v-ets erythroblastosis virus E26 oncogene homolog (ERG). Most molecular targeted therapies in other cancers are notable for their lack of serious side-effects and amazing tolerability. I hypothesized that *ERG,* the most common *ETS* oncogene found to be mutated in prostate cancer can serve as an effective molecular therapeutic target for prostate tumors. I planned to show this with novel autochthonous prostate tumor mouse models. I also hypothesized that *ERG* facilitates tumorigenesis alone or in the context of activated AKT1 by dysregulating proliferation, apoptosis and/or senescence programs *in vivo*. Demonstrating whether prostate tumors in mouse models are dependent for *ERG* for tumor survival would be the first proof of principle demonstration of molecularly targeted therapy for spontaneously arising prostate tumors *in living* animals.

The original specific aims are below:
Specific Aim#1 - Generate and characterize an inducible *ERG* prostate specific mouse model.
Rationale: I have created a novel prostate TET system mouse model and am interested in the effects of *ERG* expression alone and in combination with *AKT1* in the prostate.
Study Design: I will validate inducible expression of both *ERG* and *Luc in vivo* using real time-RT-PCR (qPCR), BLI of whole living animals and by organ Western analysis in bi-transgenic *ARR2PB-tTA*/ *ERG-tetO-Luc* (AE) mice.

Specific Aim#2 – Determine if *ERG* cooperates with *AKT1* for prostate tumorigenesis.
Rationale: *ERG* overexpression *in vitro* suggests that *ERG* may facilitate tumorigenesis, but *ERG* transgenic mouse models vary in the severity of their tumor phenotypes alone and with *AKT1* co-overexpression. The mechanism for *ERG* prostate phenotypes alone or in combination with *AKT1* overexpression *in vivo* are unknown.
Study Design: Generate *ARR2PB-tTA/MPAKT1/ ERG-tetO-Luc* (AA1E) tri-transgenic mice and compare to single oncogene mice to genetically analyze cooperation *in vivo*. Investigate using molecular techniques if *ERG* modulates proliferation, apoptosis and/or senescence programs *in vivo*.

Specific Aim#3 - Determine if *ERG* can serve as an effective molecular therapeutic target for prostate tumors *in vivo*.
Rationale: Despite the importance that *ERG* overexpression is believed to play in prostate tumorigenesis, the therapeutic value of targeting *ERG* on autochthonous prostate tumors has not been tested *in vivo*. The mechanism for any autochthonous tumor regression or stasis *in vivo* upon *ERG* inactivation is unknown.
Study Design: Following development of autochthonous prostate tumors in TET regulated mice I will treat mice with doxycycline to simulate targeted treatment against the *ERG* oncogene. Investigate using molecular techniques if *ERG* inactivation modulates proliferation, apoptosis and/or senescence programs in autochthonous prostate tumors *in vivo*.

BODY:

Progress is listed in relation to each specific task in the "Statement of Work" and highlighted by *italics* for Years 1-2 and **BOLD** font for the past year (Year 3).

Task#1 - Generate and characterize an inducible *ERG* prostate specific mouse model (months 1-17).

Numbers of mice surviving weaning and for mating: 65

1a. IACUC and other regulatory approval process for animal work (months 1-4).

As reported in our Year 1 Progress Report, we applied for and obtained approval from the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center IACUC for the studies described in our DoD grant award (see Appendix for documentation approval).

1a. Mating mice to characterize (months 4-10).

As reported in our Year 1 Progress Report, the appropriate single transgene ARR2PB-tTA (A) and ERG-tetO-Luc (E) mice were mated to produce cohorts of (AE) bitransgenic mice. There were no issues with producing the required numbers of AE mice.

We are the process of mating the new more robust prostate specific driver *Hoxb13-rtTA* mouse to produce bitransgenic, *Hoxb13-rtTA*/*ERG-tetO-Luc* (HE) and tritransgenic animals, *Hoxb13-rtTA*/*MPAKT1*/*ERG-tetO-Luc* (HA1E). We had some difficulties with sustaining this *Hoxb13-rtTA* line and have had to acquire the mice two separate times. We are now able to sustain the line and have been able to successful mate *Hoxb13-rtTA* to another inducible mouse line, *Twist1-tetO-Luc*, as a reporter to check levels of luc expression functionally with bioluminescene imaging (BLI). We see robust BLI signal from the *Hoxb13-rtTA*/ *Twist1-tetO-Luc* mice (see Fig 1 Appendix) which is much higher than what we previously observed from AE mice.

1b. Collecting tissues from AE mice to characterize ERG expression (months 8-14). AE mice will be weaned and placed on water without doxycycline and 5 males for each of the following age time points: 4, 8, 12 and 24 weeks (n=25 mice total, 5 additional for incidentals), will be interrogated using the assays mentioned below in 1d.

As reported in our Year 1 Progress Report, the appropriate numbers of AE bitransgenic mice (n=25) have been placed on drinking water without doxycycline to activate the ERG transgene.

Pending above creation of HE mice.

1c. Collecting tissues from AE mice turned OFF to characterize inducible ERG expression (months 8-14). 12 week old males will be followed for the OFF time points: 1, 2 and 4 weeks (n=20 mice total, 5 additional for incidentals) and tissues extracted for interrogation using the assays mentioned below in 1d.

As reported in our Year 1 Progress Report, the appropriate numbers of AE bitransgenic mice have been placed on regular water (n=20) for 4-6 weeks following weaning to activate the ERG transgene followed by changing to doxycycline drinking water (0.2 mg/ml) changed weekly to inactivate the ERG transgene.

Pending above creation of HE mice.

1d. Performing experiments on tissues from mice (months 14-17). Tissues from 1b and 1c above will be harvested for histology and flash frozen for molecular studies: prostate lobes, other genitourinary (GU) organs, lungs, heart, liver and spleen. These specimens will then be processed for H&E histology and immunohistochemistry (IHC) performed using anti-Myc, anti-FLAG and anti-luciferase antibodies to confirm prostate luminal cell epithelia expression. Whole lobe and organ Western blotting using the same antibodies will also be performed and transcription of *ERG* confirmed with specimens using qPCR.

See Table 1 and 2 below for summary of results. We were able to harvest as above for all the "ON" time points at least 5 mice: 4, 8, 12 and 24 weeks. Similarly, for the "OFF" time points we have been able to collect tissues from $\geq$ 5 mice from the 1, 2 and 4 week time points.

Pending above creation of HE mice.

We have performed analysis as summarized below in Table 1 & 2. The AE mice from the "ON" time points collected have had no abnormalities on gross or H&E examination of their prostates. The other organs in these mice (lungs, heart, liver and spleen) were also normal on necropsy. Similarly, the AE mice from the "ON" and "OFF" time course displayed no pathology on gross or histologic exam of the H&E slides. We have attempted IHC and westerns for protein expression of ERG that is tagged by Myc and FLAG epitope tags, but have not been able to see expression using either approach. We also attempted on a limited scale luc IHC and ERG qPCR with these samples which were similarly negative.
Pending above creation of HE mice.

1e. Analyzing results of experiments on tissues from mice (months 14-17).
See Table 1 and Tabe 2 for summary of results and "Conclusions" below for explanation of results.

Table 1 – Summary of Task #1b to date.

Genotype	4 wks On DOX	8 wks On DOX	12 wks On DOX	24 wks On DOX
AE	6 mice	7 mice	5 mice	Pending
Gross	WNL	WNL	WNL	WNL
Histologic	WNL	WNL	WNL	WNL
Myc IHC	Negative expression	Negative expression	Negative expression	Negative expression
FLAG IHC	Negative expression	Negative expression	Negative expression	Negative expression
luc IHC	ND	ND	ND	Negative expression
FLAG Western	Negative expression	Negative expression	ND	Negative expression
***ERG* qPCR**	ND	ND	ND	Negative expression

A – *ARR2PB-tTA*; DOX – doxycycline; E – *luc-tetO-ERG;* IHC – immunohistochemistry; qPCR – quantitative polymerase chain reaction; WNL – within normal limits.

Table 2 – Summary of Task #1c to date.

Genotype	1 wks Off DOX	2 wks Off DOX	4 wks Off DOX
AE	6 mice	6 mice	Pending
Gross	WNL	WNL	WNL
Histologic	WNL	WNL	WNL
Myc IHC	Negative expression	ND	ND
FLAG IHC	Negative expression	ND	ND
luc IHC	Negative expression	ND	ND
FLAG Western	Negative expression	ND	ND
***ERG* qPCR**	Negative expression	ND	ND
IHC	Negative expression	ND	ND
Western	Negative expression	ND	ND

A – *ARR2PB-tTA*; DOX – doxycycline; E – *luc-tetO-ERG;* IHC – immunohistochemistry; qPCR – quantitative polymerase chain reaction; WNL – within normal limits; ND - not done.

Each of the steps/tasks below are dependent on the steps above and have not been initiated.

Task#2 - Determine if *ERG* cooperates with *AKT1* for prostate tumorigenesis (months 14-34).

Numbers of mice surviving weaning and for mating: 150

2a. Mating mice for cooperation experiments (months 14-20).

2b. Collecting tissues from cooperation experiments (months 18-30).

2c. Performing experiments on tissues from mice (months 20-32). Tissues from 2b above will be harvested for histology and flash frozen for molecular studies: prostate lobes, other GU organs, lungs, heart, liver and spleen. These specimens will then be processed for H&E histology and IHC performed using anti-Myc, anti-FLAG and anti-luciferase antibodies. Whole lobe and organ Western blotting using the same antibodies will also be performed and transcription of *ERG* confirmed with specimens using qPCR. IHC for cleaved caspase 3 (CC3) and Ki-67. Senescence markers such as p15, p16, p21 and p27 will be analyzed by IHC and qPCR. In addition, I will perform senescence associated beta-galactosidase (SA-β-gal) staining.

2d. Analyzing results of experiments on tissues from mice (months 22-34).

Task#3 - Determine if *ERG* can serve as an effective molecular therapeutic target for prostate tumors *in vivo* (months 34-60)

Numbers of mice surviving weaning and for mating: 120

3a. Mating mice for therapeutic experiments (months 34-40).

3b. Collecting tissues from therapeutic experiments mice ON 6-12 months and then OFF 1-6 months (months 40-56).

3c. Performing experiments on tissues from mice (months 42-58). Tissues from 3b above will be harvested for histology and flash frozen for molecular studies: prostate lobes, other GU organs, lungs, heart, liver and spleen. These specimens will then be processed for H&E histology and IHC performed for Myc, FLAG, luciferase, CC3, Ki-67, p15, p16, p21 and p27. Whole lobe and organ Western blotting using the same antibodies will also be performed and transcription of *ERG* confirmed with specimens using qPCR. In addition, I will perform SA-β-gal staining.

3d. Analyzing results of experiments on tissues from mice (months 44-60).

KEY RESEARCH ACCOMPLISHMENTS:

- *Generation of possibly inducible bitransgenic prostate specific ERG expressing mice.*
- *Characterization of inducible regulation of this transgenic ERG model system.*
- *Confirmation that our ARR2Pb-tTA mouse line is not robust enough to drive expression of tetO-regulated genes in the mouse prostate.*
- **Initiating development of novel bitransgenic, *Hoxb13-rtTA*/*ERG-tetO-Luc* (HE) and tritransgenic animals, *Hoxb13-rtTA*/*MPAKT1*/*ERG-tetO-Luc* (HA1E) using the more robust prostate specific driver *Hoxb13-rtTA*.**

REPORTABLE OUTCOMES:

- During this first year of support we have not published any manuscripts, abstracts or presented this work at any venue other then at our own private lab meetings.
- No licenses were applied for.
- No degrees were obtained that are supported by this award.
- We did not develop any cell lines or serum repositories, but tissues from our AS mice were banked for further analysis as described above in the "**Body**" section.
- No infomatics databases were constructed, but a novel animal model was developed that we are trying to characterize as above in the "**Body**" section.
- No additional funding was applied for based on this work
- No employment or research opportunities applied for and/or received based on experience/training supported by this award.

CONCLUSION:

During this third year of support we have not been able to been able to adhere to the timeline of our "Statement of Work" - Task#2 - Determine if *ERG* cooperates with *AKT1* for prostate tumorigenesis (months 14-34) or Task#3 - Determine if *ERG* can serve as an effective molecular therapeutic target for prostate tumors *in vivo* (months 34-60). We were previously successful at completing the tasks for Task#1 - Generate and characterize an inducible *ERG* prostate specific mouse model (months 1-17), but this characterization of ERG expression from our old prostate inducible mouse model, *ARR2PB-tTA*, did not demonstrate any detectable prostate specific ERG expression at the protein level using Western or IHC (see Tables 1 & 2 above). However, characterization of the ERG founder lines indicated that expression was feasible using a different promoter element driving a similar tTA gene in the liver (see Fig 2 Appendix). This was also indirectly confirmed with another rtTA mouse line CMV-rtTA (C) where inducible expression is ubiquitous (data not shown).

We had in our Year 2 progress report given possible explanations for the lack of a phenotype despite prostate epithelium specific expression of other tetO reporter lines include (1) the level of *ERG* expression is in sufficient as driven from the *ARR2PB-tTA* line; and/or (2) more time is required to develop a phenotype. Briefly, our data led us to the conclusion that the level of expression from the *ARR2PB-tTA* line was too low and perhaps insufficient for the *in vivo* experiments described in our proposal. To remedy this issue with low prostate specific expression we proposed to re-start Task #1 of the project with the new prostate specific TET driver mouse, *Hoxb13-rtTA* (H) (4), in collaboration with Dr. Charles Bieberich. The *Hoxb13-rtTA* line allows for much more robust expression of tetO target genes than our *ARR2PB-tTA* line (see Fig 1 Appendix). The breeding between our *tetO-ERG* mice and Dr. Bieberich's *Hoxb13-rtTA* mice over the past year has been problematic (see above). Briefly, we had difficulties with breeding the *Hoxb13-rtTA* line but have overcome these issues and have been able to see robust prostate specific BLI signal using this *Hoxb13-rtTA* driver with another inducible reporter mouse line (see Fig 1 Appendix); which was much higher than what we previously observed from AE mice. We are now in the process of reinitiating our studies on the ability of ERG to collaborate with *AKT1* with these new mice, *Hoxb13-rtTA*/ *tetO-ERG* (HE). Thus we are still optimistic that our tetO-ERG lines are capable of inducible prostate specific ERG expression but ultimately are going to use a newly reported TET inducible prostate mouse model, *Hoxb13-rtTA* (4).

"So What"

Despite the importance that *ERG* overexpression is believed to play in prostate tumorigenesis, the therapeutic value of targeting *ERG* rearrangements has not been tested *in vivo*. The ability to interrogate using *in vivo* model systems whether *ERG* or other oncogenes are good molecular therapeutic targets could provide a huge leap forward for prostate cancer research and treatment of prostate cancer patients. Demonstrating whether prostate tumors in my inducible transgenic mice are dependent for *ERG* for tumor maintenance would be the first proof of principle demonstration of molecularly targeted therapy for prostate tumors *in vivo* and we will be able to determine whether molecularly targeted therapy against *ERG* in the context of activated *AKT1* would be an effective therapy for prostate tumors.

REFERENCES:

1. A. Jemal *et al.*, *CA Cancer J Clin* **59**, 225 (Jul-Aug, 2009).
2. C. Kumar-Sinha, S. A. Tomlins, A. M. Chinnaiyan, *Nature reviews* **8**, 497 (Jul, 2008).
3. S. A. Tomlins *et al.*, *Science* **310**, 644 (Oct 28, 2005).
4. V. Rao *et al.*, *Prostate*, (Feb 1, 2012).

APPENDIX:

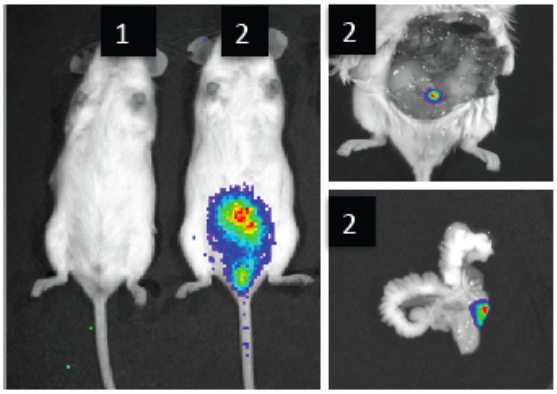

Fig 1. ***Generation of an inducible luc prostate epithelial specific mouse model.*** *Mice containing a prostate specific TET driver transgene, Hoxb13-rtTA was crossed with a reporter mouse luc-tetO-Twist1 line to produce bi-transgenic animals (HT). The presence of doxycycline allows the rtTA protein to bind and activate the tetO promoter. Removal of doxycycline triggers a conformational change which prevents tetO binding, activation and inhibits Twist and luc transcription. HT animals express luciferase inducibly in the prostate as shown by bioluminescence imaging* (BLI) *(ip injection with luciferin substrate and imaged 10 minutes later on a Xenogen Spectrum machine shows a colored bright region in the lower abdomen/high pelvis). Dox – doxycycline was given to animals in the drinking water [2 mg/ml]. Animal 1 has a Hoxb13-rtTA genotype and animal 2 is an HT mouse. The smaller panels on the right are animal 2 after necropsy and dissection of the prostate and seminal vesicles. In these right panels prostate inducible and specific luc expression can be seen by BLI.*

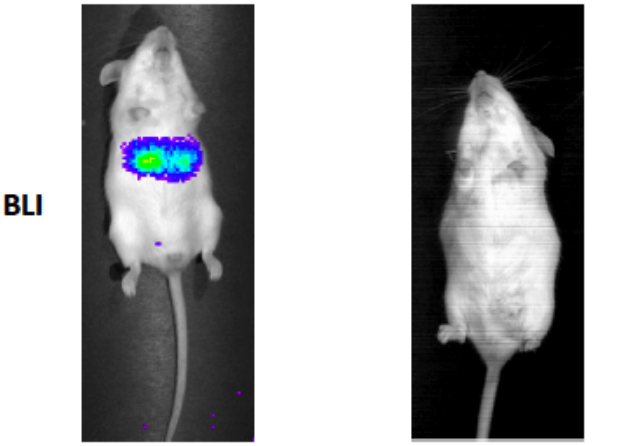

Fig 2. ***Generation of an inducible luc liver epithelial specific mouse model.*** *Mice containing a liver specific TET driver transgene, LT2-tTA was crossed with a reporter mouse luc-tetO-ERG line to produce bi-transgenic animals (LE). The absence of doxycycline allows the tTA protein to bind and activate the tetO promoter. Addition of doxycycline triggers a conformational change which prevents tetO binding, activation and inhibits ERG and luc transcription. LE animals express luciferase inducibly in the liver as shown by bioluminescence imaging* (BLI) *(ip injection with luciferin substrate and imaged 10 minutes later on a Xenogen Spectrum machine shows a colored bright region in the right upper abdomen). Dox – doxycycline was given to animals in the drinking water [0.04 mg/ml].*

JOHNS HOPKINS
UNIVERSITY

Animal Care and Use Committee

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To: Dr. Phuoc Tran
Department of Oncology

From: Nancy A. Ator, Ph.D.
Chair, Animal Care and Use Committee

Date: 12/10/2010

Subject: Amendment Approval Memo

On 12/09/2010, the Johns Hopkins University Animal Care and Use Committee (ACUC) approved the following [Procedures] amendment to your research protocol. A copy of the approved amendment is attached.

Protocol Number: MO09M331

Title: Transgenic models of oncogene induced tumorgenesis and organ fibrosis

Expiration Date: 08/21/2011

Additional modifications to this protocol can be requested by submitting the appropriate amendment form (i.e., Change in Animal Number, Change in Personnel, or Change in Procedures) to the ACUC office for review and approval. Copies of all current forms can be found on our website: www.jhu.edu/animalcare.

For guidance on protocol modifications that require amendments, please refer to the reverse side of this letter. If the locations for outside housing or procedures change, please submit a Change in Location Form, also available on the website.

Johns Hopkins University Animal Care and Use Committee

CHANGE IN PROCEDURE(S) OR ANIMAL NUMBERS AMENDMENT REQUEST FORM

Release date: 12/08

Below for ACUC Use	
Date Received:	11/18/10
Expiration Date:	8/21/11
☑ Logged	☑ Database

Protocol Number: MO09331

Protocol Title: Transgenic Models of Oncogene Induced Tumorigenesis and Organ Fibrosis

Principal Investigator: Phuoc T. Tran

Department: Radiation Oncology School: SOM

Building: CRB2 Room: B406 Campus: East Baltimore

Office Phone: x43880 Fax: x22821 E-mail: tranp@jhmi.edu

If this request is being faxed or emailed (with an electronic signature) to the ACUC Office, an original is not needed.

Please indicate which changes you are requesting by an **X** next to each category below. Describe the change(s) and reasons on page 2 of this form. Please return a **signed copy** of this form to the ACUC Office, Reed Hall, room B122 or fax to 443-287-3747 (7-3747).

To add new personnel or change the PI, please complete the **Change in Personnel Amendment Request Form** or **Change in PI Amendment Request Form.**
To change a location for animal use complete the **Change in Location Form.**
All forms are available on the web at www.jhu.edu/animalcare/forms1.html.

____ **Modify anesthetic or analgesic agents:** *State the name of the agent, dose or dose range, route of administration and frequency range for any drug to be added. Previously approved agents will remain on the protocol. If you need to withhold analgesia, indicate the reasons why and see "Modify Pain Category" below to see if it applies.*

____ **Modify Euthanasia:** *Describe any changes in the method of euthanasia (be sure proposed method is in compliance with the 2007 AVMA Guideline on Euthanasia, which can be viewed at www.avma.org/resources/euthanasia.pdf)*

__X__ **Modify Procedures:** *Provide a complete description and rationale for the proposed experimental changes. Indicate if they will change the degree of invasiveness of a procedure or discomfort to the animal. (i.e., the withholding of analgesics; change from non-survival to survival surgery; change in number, duration, or frequency of procedures performed on the animal, etc.). See "Modify Pain Category" below to determine if it applies.*

____ **Modify Surgical Procedures:** *Describe any changes to approved surgical procedures.*

____ **Modify Radiation; or Radioactive, Infectious or Biohazardous Agent:** *Provide rationale for adding this new agent, list all necessary safety precautions, and describe any modifications you plan to make to your currently-approved procedures. Attach pertinent approval letter or copy of application from Health, Safety & Environment as appropriate).*

____ **Modify Animal Numbers:** *Indicate the number of **additional** animals you are requesting that will fall under each pain category in the chart below. Provide a justification for the change in animal numbers. Each animal should be categorized only once. If adding animals or procedures to category D or E for the first time, please see "Modify Pain Category" below.*

Number Requested	*Pain Category*
	B Breeders
	C No pain or distress
	D Alleviated Pain or distress
	E Unalleviated Pain or distress

_____ **Modify Pain Category:** *Please describe the changes that will affect the pain category. If adding animals or procedures to category D or E for the first time, please include a description of what alternatives to procedures that may cause more than momentary or slight pain or distress have been considered and why no alternative was selected. See questions 17b-e on the full protocol form for the information that should be included with respect to category D or E procedures.*

_____ **Add Satellite Housing:** *Include Satellite Housing amendment with this form*

_____ **Other:** *describe on page 2.*

CHANGE IN PROCEDURE(S) AMENDMENT REQUEST FORM

Describe the requested change(s) following the guidelines for the specific modification as per page 1 of the form (attach additional pages as necessary).

To determine the role of oncogenes, such as ERG, for tumorigenesis and tumor maintenance using the Tet system.

Justification: Tumorigenesis is thought to involve multiple steps many of which are determined by changes in specific genes. Studies have demonstrated that oncogenes are causative in tumorigenesis. Oncogenes are also involved during normal developmental processes where cells acquire increased migratory abilities enabling cells to form the many and varied organs of the body. Dysfunctional oncogene expression has been implicated in both tumorigenesis and tissue fibrosis. The Tran laboratory is interested in understanding the role of various oncogenes, including but not limited to Twist1, hSNAI1 and ERG, in the processes of tumorigenesis, tumor maintenance and tissue fibrosis using mice that express oncogenes. In most cases, the expression of these oncogenes will be induced or turned "ON" and "OFF" using the tetracycline (or doxycycline) regulatory system (TET system).

Development of imaging surrogates for use in localization and monitoring treatment of tumors and organ fibrosis in living rodent subjects has been previously described in approved amendments. Many of the animal models we use are transgenic models (knock in, knock out) that recapitulate human disease. There are no computer simulations that serve this purpose.

We hypothesize that serial non-invasive imaging followed by confirmation with histopathology will allow our group to monitor the development of tumors and track tumor regression in our cohort of transgenic mouse models using the Tet system.

1) To use non-invasive serial imaging studies and standard histo-pathological analysis to monitor tumorigenesis using the Tet system. We will determine if expression of oncogenes alone or in conjunction with previously approved agents and other oncogenes enhance tumorigenesis and/or lung fibrosis in the mice models as a part of our already approved protocol by providing the animals doxcycline in their water or chow as (MO09M331).

Cohorts of weaned, age-matched, control and experimental mice will be devoid of doxycycline or placed on doxycycline (depending on the transgenic model) in their drinking water to activate expression of an *oncogene* being studied. Mice will be monitored weekly for symptoms of morbidity as stated below. Prior experience with a separate luciferase tagged primary *Twist1* tumor model indicates that biolumeniscence imaging (BLI) signal correlates with tumor burden. Therefore, cohorts with the *Luc* reporter will also be followed for tumor development non-invasively by use of serial BLI (using our already approved imaging amendment) and correlated with disease pathology following necropsy at defined periods. Based on prior literature and our experience mice from each cohort will be sacrificed at time points of between 0-18 months of age depending on physical and imaging findings. These animals will be processed at necropsy for prostate lobes, other genitourinary (GU) organs, lungs, heart, liver and spleen and these specimens will be harvested for histology and flash frozen for molecular studies.

2) To use non-invasive serial imaging studies and standard histo-pathological analysis to monitor tumor maintenance using the Tet system.

Following development of autochthonous tumors in TET regulated oncogene mice as determined by serial imaging and from my time course studies above, we will treat mice with doxycycline to simulate targeted treatment against the tetO-regulated oncogenes.

Tumor moribund mice that are known have tumors from imaging or suspected based on time course experiments above will be injected intraperitoneally with 100 micrograms of doxycycline in PBS and then restricted to water containing doxycycline changed weekly (or depending on the system normal water free of doxycycline). Cohorts of tumor morbid mice following oncogene inactivation will be followed by weekly inspection and imaging. At defined periods of between 0-12 month animals will serially imaged and sacrificed and necropsies and tumor analysis performed as above; or before if euthanasia is required for humane reasons.

All animals will be monitored and euthanized immediately if they exhibit the following symptoms:

- Ulceration and bleeding of the tumor
- Anorexia indicated by the absence of feces in cage
- Does not drink water leading to dehydration evidenced by tenting of the skin
- Hunched up, unwilling to move, favoring a limb or guarding the incision site
- Failure to groom reflected in a ruffled or dirty coat
- Excessive licking/scratching, redness and swelling at incision site, and self-mutilation
- Aggressive behavior especially when attempting to pick up the animal
- Squealing, struggling, twitching, tremors, convulsions, weakness
- Panting, labored breathing, reddish-brown nasal/ocular discharge
- Cold or blue extremities (hypothermia) or hot or red extremities (hyperthermia)

I understand that these changes must not be implemented until I receive approval for the changes from the Animal Care and Use Committee.

PI Signature: _____ Date: 11/18/2010

IACUC Chair's Signature: Nancy A. Aton Date: 12/9/10